STUDY TITLE: Static, Acute, 48-Hour Toxicity Screening Test with *Daphnia magna*

AUTHOR:

ORIGINAL REPORT COMPLETED: September 21, 2010

REPORT REVISION 1 COMPLETED: November 23, 2010

PERFORMING LABORATORY:

LABORATORY PROJECT ID:

WORK REQUEST NUMBER:

SERVICE CODE NUMBER:

SPONSOR:

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Work Completed by: ___ _____ 23 Nov 2010
Date

Reviewed by: ____ _____ 23 NOV 2010
Date

Issued by Study Director: ____ _____ 23 Nov 2010
Date

STUDY INFORMATION

Substance Tested:	
Number:	
Composition:	
Purity:	See composition, above
Physical Characteristics:	Amber liquid
Stability:	The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Study Initiated/Completed:	August 24, 2010 / (see report cover page)
Experimental Start/Termination:	August 24, 2010 / August 26, 2010
In-Life Initiated/Completed:	August 24, 2010 / August 26, 2010
Notebook Number(s):	

REASON FOR REVISION

Study Information page was revised at the request of the sponsor, and a Records and Sample Storage section was added.

SUMMARY

The acute toxicity of to the cladoceran, *Daphnia magna* (less than 24 hours old) was determined in an unaerated, 48-hour, static test.

The study was conducted with four concentrations of and a dilution water control at a mean temperature of 20.4°C (range of 19.8-21.0°C). One test chamber was used per test concentration with 10 test organisms in each chamber. Based on visual observations, the dilution water control and 0.6, 6.0, 60, and 600 mg/L test concentrations were clear and colorless with no visible precipitate at test start. All water quality parameters were within acceptable limits during the exposure.

Exposure of daphnids to the dilution water control and nominal, total formulation concentrations of 0.6, 6.0, 60, and 600 mg/L [corresponding to nominal active substance (a.s.) concentrations of 0.12, 1.2, 12, and 120 mg a.s./L] resulted in 0, 0, 0, 0, and 0% immobility, respectively, at the end of 48 hours. No immobility or sublethal effects were seen in the dilution water control test organisms at test end. The highest nominal concentration causing no immobility at test end was 600 mg/L (120 mg a.s./L). The lowest nominal concentration causing 100% immobility at test end was greater than 600 mg/L (120 mg a.s./L). Nominal concentrations were used to calculate the 48-hour EC_{50} value.

The results are summarized as follows:

Nominal concentrations of mg/L	dilution water control, 0.6, 6.0, 60, and 600
Nominal a.s. concentrations, mg a.s./L	dilution water control, 0.12, 1.2, 12, and 120
48-hour EC_{50} for based on nominal concentrations, mg/L	Greater than 600
48-hour EC_{50} for a.s. based on nominal concentrations, mg a.s./L	Greater than 120

exhibited low concern for aquatic hazard in an unaerated, 48-hour, static acute test using the cladoceran, *Daphnia magna* (less than 24 hours old).

MATERIALS AND METHODS

A. Test Solution Preparation

The 6.0, 60, and 600 mg/L test substance solutions were prepared by adding the appropriate amount of to well water ( ) in 1-L beakers and stirring for approximately 20 minutes. The 0.6 mg/L test substance solution was prepared by diluting the appropriate volume of the 60 mg/L solution with additional in a 1-L beaker. All test solutions and the dilution water control were then stirred for an additional 15 minutes. Based on visual observations, the dilution water control and 0.6, 6.0, 60, and 600 mg/L test concentrations were clear and colorless with no visible precipitate at test start.

B. Dilution Water

Dilution water originated from the well, which is 480-feet deep and is cased and sealed to bedrock. The hardness of the is adjusted to approximately 100-140 mg/L as $CaCO_3$ by the flow-proportioned addition of $CaCl_2$. The is then aerated, passed through a green sand filter to remove iron, and filtered through 50-, 10-, and 3-µm filters to remove particulates. The water is heated or chilled as appropriate and distributed through aged polyvinyl chloride piping. The dilution water is analyzed at least once yearly for major anions and cations, metals, total organochlorine and organophosphate pesticides, and polychlorinated biphenyls. The dilution water meets OECD[1] and ASTM[2] specifications.

C. Test Organism Culture

Daphnia magna were reared at in 250-mL (1 per beaker) or 1000-mL glass beakers (10 per beaker at culture initiation) which contained 200 mL or 1000 mL of aerated, filtered held at approximately 20°C. Daphnids were fed on a daily basis with 3 mL/L of a yeast, cereal leaves and trout chow (YCT) mixture (standardized to 1700 to 2100 mg/L total solids) and the green alga, *Pseudokirchneriella subcapitata*, at a rate of approximately 62,500 cells/mL of culture medium. The combination of YCT and alga is equivalent to approximately 0.1-0.2 mg total organic carbon per daphnid. Neonates used in this test were less than 24 hours old and were collected from the 4th brood of 14-day old parent daphnids. Sickness, injury, and abnormalities were not seen and ephippia were not being produced by the parent daphnids. No adult immobility was seen in the cultures used for testing during the 48-hour pretest period. *Daphnia magna* were identified by labels on the culture beakers and test chambers.

D. Test Methods

Four nominal concentrations and a dilution water control were used in this study. The nominal concentrations were 0.6, 6.0, 60, and 600 mg/L which corresponded to 0.12, 1.2, 12, and 120 mg a.s./L.

Glass beakers (1000-mL) containing 1000 mL of test solution (approximately 12.9-cm test solution depth) were used as test chambers. One replicate test chamber was used per test

concentration with 10 daphnids in each chamber. The test chambers were covered with a glass plate during the test.

Daphnia magna neonates, less than 24 hours old, were used in this study. Daphnids were not fed during the test. Addition of daphnids to test solutions was initiated after mixing of the test solutions was completed. Immobility and behavioral observations were made daily. The criterion for the effect (immobility) was a lack of reaction to application of a gentle stimulus.

A recirculating waterbath was used to maintain mean temperature in the test chambers during the 48-hour test at approximately 20.4°C with a range of 19.8 to 21.0°C. A photoperiod of 16 hours light (approximately 165 to 186 Lux) and 8 hours darkness was employed which included 30 minutes of transitional light (8 to 15 Lux) preceding and following the 16-hour light interval.

Dissolved oxygen concentration, pH, and temperature were measured in all replicates of the dilution water control and test substance concentrations. These measurements were taken before daphnids were added at test start and at test end. Test solutions were not aerated during the test and were disposed of in an appropriate manner at test end.

RESULTS AND CONCLUSION

Exposure of daphnids to the dilution water control and nominal concentrations of 0.6, 6.0, 60, and 600 mg/L (equivalent to 0.12, 1.2, 12, and 120 mg a.s./L) resulted in 0, 0, 0, 0, and 0% immobility, respectively, at the end of 48 hours. No immobility or sublethal effects were seen in the dilution water control test organisms at test end. The highest nominal concentration causing no immobility at test end was 600 mg/L (120 mg a.s./L). The lowest nominal concentration causing 100% immobility at test end was greater than 600 mg/L (120 mg a.s./L). Nominal concentrations were used for calculation of EC_{50} values.

The 48-hour EC_5 for based on nominal concentrations and immobility, was greater than 600 mg/L. exhibited low concern for aquatic hazard[3] in an unaerated, 48-hour, static acute test using the cladoceran, *Daphnia magna* (less than 24 hours old).

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at ,

or

REFERENCES

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for the Testing of Chemicals: 202, 13 April 2004.

2. American Society for Testing and Materials (ASTM). (1988). Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E 729-88a. Annual Book of ASTM Standards, Vol. 11.04.

3. Smrchek, J., Clements, R., Morcock, R., and Rabert, W. (1993). Assessing ecological hazard under TSCA: methods and evaluation of data. Environmental Toxicology and Risk Assessment, ASTM STP 1179. (W.G. Landis, J.S. Hughes, and M.A. Lewis, Eds.), pp 22-39. American Society for Testing and Materials, Philadelphia.

Table 1
Dissolved Oxygen and pH of Test and Dilution Water Control Solution Samples

Nominal Concentration (mg/L)	0 Hours		48 Hours	
	Dissolved Oxygen (mg/L)	pH	Dissolved Oxygen (mg/L)	pH
Dilution Water Control	8.7	8.1	9.1	8.0
0.6	8.8	8.1	9.1	8.1
6.0	8.8	8.1	9.1	8.1
60	8.9	8.1	9.1	8.1
600	8.9	8.2	9.1	8.2

Table 2
Immobility of *Daphnia magna* in an Unaerated, Static, Acute, 48-Hour Test with

Nominal Concentration (mg/L)	Number Immobile / Number at Study Start	
	24 Hours	48 Hours
Dilution Water Control	0/10	0/10
0.6	0/10	0/10
6.0	0/10	0/10
60	0/10	0/10
600	0/10	0/10